Characteristics and Clinical Implications of Carbapenemase-Producing *Klebsiella pneumoniae* Colonization and Infection, Italy

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Learning Objectives

Upon completion of this activity, participants will be able to:

- Describe epidemiology of KPC-*Kp* and molecular characterization of KPC-*Kp* strains in colonized and infected inpatients with mild (MI) or serious (SI) infections in Italy, according to a multicenter cohort study of 1,071 patients with KPC-*Kp*
- Determine clinical characteristics and outcomes of KPC-*Kp* in colonized and infected inpatients with MI or SI in Italy, according to a multicenter cohort study
- Identify treatment and other clinical implications of KPC-*Kp* in colonized and infected inpatients with MI or SI in Italy, according to a multicenter cohort study

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Klebsiella pneumoniae carbapenemase–producing *K. pneumoniae* (KPC-*Kp*) has been endemic in Italy since 2013. In a multicenter cohort study, we investigated various aspects of KPC-*Kp* among patients, including 15-day mortality rates and delays in adequate therapy. Most (77%) KPC-*Kp* strains were sequence types ST512 or ST307. During 2017, KPC-*Kp* prevalence was 3.26 cases/1,000 hospitalized patients. Cumulative incidence of KPC-*Kp* acquired >48 hours after hospital admission was 0.68% but varied widely between centers. Among patients with mild infections and noninfected colonized patients, 15-day mortality rates were comparable, but rates were much higher among patients with severe infections. Delays of ≥4 days in receiving adequate therapy more frequently occurred among patients with mild infections than those with severe infections, and delays were less common for patients with known previous KPC-*Kp* colonization. Italy urgently needs a concerted surveillance system to control the spread of KPC-*Kp*.

The global emergence and spread of carbapenem-resistant *Enterobacteriaceae* (CRE) pose a major health threat, causing severe illness and high healthcare costs (*1*). Infections caused by CRE also are associated with high mortality rates because extensive resistance to so-called last-line antimicrobial drugs, such as carbapenems, limit the treatment options (*2*–*5*). Only a few antimicrobial drugs, such as colistin, fosfomycin, tigecycline, and ceftazidime/avibactam, are effective against CRE. Moreover, the remaining therapeutic options often have high toxicity profiles, and rates of resistance to these antimicrobial drugs already are increasing (*6*).

In a 2014 study conducted by the European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) Working Group, 455 sentinel hospitals in 36 countries submitted clinical isolates (*7*). Among the 2,703 isolates submitted, 2,301 (85%) were *Klebsiella pneumoniae* and 402 (15%) were *Escherichia coli*, including samples identified as carbapenemase producers among 850 (37%) *K. pneumoniae* and 77 (19%) *E. coli* isolates. Identified carbapenemase-producers included 4 gene families: *K. pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase, oxacillinase 48-like, and Verona integron-encoded metallo-β-lactamase (*7*). Positive clinical specimens were found in 1.3 patients/10,000 hospital admissions, but prevalence differed greatly between countries and the highest rates were registered in countries in the Mediterranean and Balkan regions (*7*). Among these countries, Italy, Greece, and Romania reported the highest percentages of carbapenem resistance. In addition, CRE rates increased from 15% in 2010 to 36% in 2016 (*8*–*10*), and CRE became endemic in Greece in 2010 and Italy in 2013 (*11*). Nevertheless, currently published information is too scant to define the complete picture of KPC *K. pneumoniae* (KPC-*Kp*) epidemiology in both clinical isolates and surveillance screening samples (*12*).

In this context, we set up a network of 15 hospitals in Lombardy, the most populous region in Italy, and established a cohort of patients affected by KCP-*Kp*. The overarching goal of the KPC-*Kp* Study Group was to identify the challenges of controlling the spread of the bacterium. We describe KPC-*Kp* epidemiology, treatment, and in-hospital mortality rates, along with molecular characterization of KPC-*Kp* strains in colonized and infected inpatients.

Methods

Study Design, Setting, and Patients

We conducted a multicenter cohort study during June 2016–April 2018, which included 15 hospitals in Lombardy (Figure 1; Appendix, https://wwwnc.cdc.gov/EID/article/27/5/20-3662-App1.pdf). We asked each enrolled hospital to include data on all consecutively hospitalized adult patients who had ≥1 positive KPC-*Kp* isolate during their hospital stay. For patients hospitalized multiple times during the study period, we only considered the first hospitalization. For centers including patients during 2017, the year for which we had a full 12 months of data, we retrieved the administrative datasets of all admitted patients (Figure 1). We merged these data with

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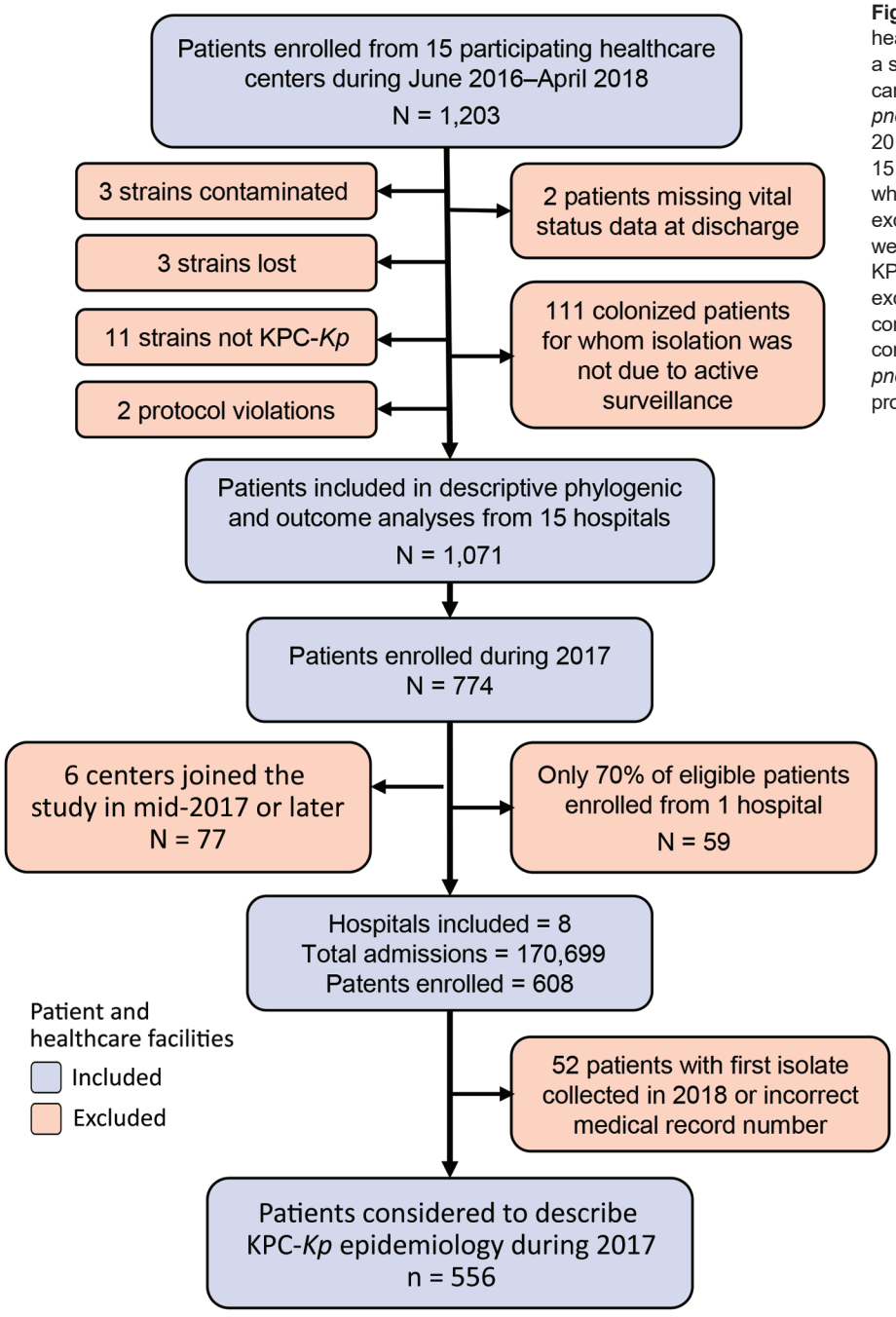

Figure 1. Flow chart of network of healthcare centers participating in a study of *Klebsiella pneumoniae*–carbapenemase producing *K. pneumoniae* (KPC-*Kp*), Italy, 2016–2018. The KPC-*Kp* network included 15 hospitals. Patients were included when KPC-*Kp* was diagnosed and excluded for various reasons. Hospitals were included when they submitted KPC-*Kp*–confirmed isolates and excluded from analysis when had no confirmed patients or did not enroll all confirmed patients. KPC-*Kp*, *Klebsiella pneumoniae*–carbapenemase producing *K. pneumoniae*.

those available in the KPC-*Kp* patient cohort database and used the combined dataset to describe KPC-*Kp* epidemiology in the hospitalized population.

The study protocol was first approved by the Research Ethics Committee of the coordinating center, Ospedale San Gerardo (Monza, Italy). Informed consent requirement was waived due to the study's observational, noninterventional design. The study protocol was subsequently approved by the ethics committees of the 14 other participating centers. In accordance with local ethics committee requirements, 3 centers did not waive informed consent. Because this was an observational study, treatment for KPC-*Kp* infections was at the discretion of the attending physicians and no change to the center-specific surveillance protocol was required.

In all centers, intensive care unit (ICU) patients were tested for CRE at admission and weekly through rectal swab specimens or other surveillance cultures. The same protocol was applied heterogeneously in

hospital wards in which patients are considered to be at higher risk of acquiring CRE, such as hematology, solid organ transplant, and geriatric units (Appendix Table 1). For the other wards, most centers performed surveillance rectal swab specimens at admission on the basis of major risk factors for CRE, such as previous CRE colonization, previous hospitalization during the 12 months before inclusion, or both. Of note, only 3 of the 15 participating centers, B, C, and I (Appendix Table 1), combined the 2 surveillance strategies described for specific wards and patients at higher risk of acquiring CRE.

Patient Classification

Patients were classified according to the most clinically relevant KPC-*Kp* isolate collected from them between hospital admission and discharge. Thus, for patients whose first isolate was attributable to colonization and a subsequent isolate was attributed to an infection, only the second isolate was considered. We used US Centers for Disease Control and Prevention criteria (*13*) to define diagnosed infection and diagnosis was confirmed by an infectious disease specialist. Infections were classified as KPC-*Kp* bacteremia when a blood culture was positive for a KPC-*Kp* strain with or without KPC-*Kp*–positive cultures from ≥1 other site and the patient had clinical signs of systemic inflammatory response syndrome requiring antimicrobial drug treatment. We defined nonbacteremic KPC-*Kp* infections by documented recovery of a KPC-*Kp* isolate from nonblood cultures, such as intra-abdominal wounds, urine, or bronchoalveolar lavage fluid; absence of KPC-*Kp*–positive blood culture during the index hospitalization; and clinical signs of infection.

In line with other studies (*14*), we classified KPC-*Kp* cases according to infection severity. We classified cases of KPC-*Kp* bloodstream or lower respiratory tract infections, and clinical presentation of septic shock, regardless of infection site, as severe infections. We classified infections from the urinary tract, surgical wounds, or other sites without septic shock as mild infections. We classified all cases identified through active surveillance as colonized when ≥1 culture sample grew KPC-*Kp* but the patient did not develop KPC-*Kp* infection during hospitalization.

Data Collection

For patients included in the KPC-*Kp* cohort, data were entered into the web-based case form after pseudonymization of personal data. Data were collected on demographic characteristics, medical history, underlying diseases, previous hospitalization, previous KPC-*Kp* infection, surgery ≤30 days before KPC-*Kp* isolation, invasive procedures ≤72 hours before KPC-*Kp* isolation, antimicrobial drug therapy ≤30 days before KPC-*Kp* isolation, dates of admission to hospital, and ward of isolation. Date of hospital discharge and patient status at discharge also were collected. The date and ward where the patient was hospitalized when KPC-*Kp* was isolated, the source of isolation, and resistance spectrum also were collected and entered into the web-based case record form. Antimicrobial treatment, including empirical treatment and post-antibiogram treatment regimen, were recorded. Empirical treatment was defined as adequate when it included ≥1 antimicrobial drug with in vitro activity against the KPC-*Kp* isolate. Data were collected in a web-based case report form.

For enrolled centers submitting patient data during 2017, we retrieved the clinical record datasets of all admitted patients after pseudonymization of personal information. To verify centers included all eligible patients, we retrieved the total number of patients with ≥1 KPC-*Kp*–positive isolate registered in the microbiology laboratory of each center and compared that with the total number of patients included in the cohort (Appendix).

Microbiology and Genomic Analysis

The clinical microbiology laboratory of each of the 15 participating centers performed isolate identification and routine antimicrobial susceptibility testing (Appendix). CRE was defined by using Clinical and Laboratory Standard Institute guidelines (*15*). All bacterial strains were sent to a central microbiological laboratory at Ospedale San Raffaele for whole-genome sequencing (Appendix).

Statistical Analysis

We estimated the prevalence of KPC-*Kp* in hospitalized patients in the region of Lombardy during 2017, the cumulative incidence of acquired KPC-*Kp* infections among hospitalized patients, and the cumulative incidence of acquired KPC-*Kp* infections occurring >48 hours after hospital admission among hospitalized patients in the same region. We calculated and reported crude estimates for all centers and estimates standardized by age and ward of isolation (Appendix).

To study the role of KPC-*Kp* infection severity on 15-day mortality rates, we considered a multivariable Cox proportional hazard model and the related hazard ratio (HR) estimates and adjusted by center for a random effect and number of days from hospitalization to KPC-*Kp* isolation. Colonized patients

frequently have shorter hospital stays than infected patients. Because a shorter discharge time could affect our results, we performed a sensitivity analysis in which we excluded early-discharge patients. We performed a subgroup analysis to quantify excess mortality hazard due to septic shock among patients with bloodstream infections (Appendix).

We used multivariable mixed logistic regression models and accounted for clustering at the center level to evaluate the association between patient characteristics and delayed or inadequate empirical therapy, which we considered as outcome variables. We adjusted the models for age and type of KPC-*Kp* infection.

Results

Center Characteristics

Among all centers, the median number of annual admissions was 27,600 (interquartile range [IQR] 18,287–40,000). Among 15 enrolled centers, 9 (60%) maintained enrollment over 12 consecutive months; centers had a mean enrollment duration of 13.8 months (Appendix Figure 1).

Patient Baseline Characteristics

Among 1,203 consecutive KPC-*Kp*–positive hospitalized patients found during study, 89.0% (1,071) were considered in the analyses and 11% (132) were excluded for various reasons (Figure 1).

The median age among patients was 72 (IQR 61–80) years, 65% were male, and 35% were female; KPC-*Kp* was isolated from 275 (25.7%) ICU patients (Table 1). Among patients in the study cohort, >90% had ≥1 underlying condition, 40% of whom had congestive heart failure, peripheral vascular disease, or chronic renal failure. Severe infections were diagnosed in 221 (20%) patients and mild infections in 109 (10%) patients. Colonized patients n = 741, 69.2%), had a median of 6 days between hospitalization and KPC-*Kp* isolation, which was much lower than for patients with severe (median 12 days) or mild (median 11 days) infections. Bloodstream infections accounted for 54% of all infections, and rectal swab samples accounted for 67% of all colonizations (Appendix Figure 2).

Distribution, Phylogeny, and Resistance Mechanisms of KPC-*Kp* Clones

Among the 1,071 patient strains isolated, 82 were from colonized patients included at the end of April 2018; these samples did not arrive at the central laboratory in time for genotyping. Of the 989 strains analyzed, 32 different sequence types (STs) were identified. The most numerous clones were ST512 in 45% (441), ST307 in 33% (326), ST258 in 7% (71), and ST101 in 6% (57) of isolates (Appendix Table 3). We identified 2 KPC variants, KPC-2 and KPC-3, in 68% of isolates. KPC-2 was absent in ST512 but predominant in ST307 and ST258. Core-genome, single-nucleotide polymorphism (SNP) analysis revealed that ST512 was scattered across all centers, but ST307 was represented in smaller, more localized clusters (Figure 2; Appendix Table 3).

Table 1. Characteristics of patients identified in multicenter surveillance for *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae*, Italy*

Characteristics	KPC-*Kp* patients, n = 1,071
Sex	
M	694 (64.8)
F	377 (35.2)
Median age (IQR)	72 (61–80)
Ward of isolation	
Intensive care unit	275 (25.7)
Infectious diseases	81 (7.6)
Surgery	149 (13.9)
Geriatrics	47 (4.4)
Oncology	34 (3.2)
Hematology	42 (3.9)
Other medical wards	443 (41.4)
KPC-*Kp* colonization in previous 12 mo	333 (31.1)
Hospitalization in previous 12 mo	865 (80.8)
Antimicrobial therapy in the 30 d before hospitalization	782 (73.0)
Major surgery in the previous 30 d	262 (24.4)
Underlying conditions†	989 (92.3)
Congestive heart failure	192 (17.9)
Peripheral vascular disease	197 (18.4)
Cerebrovascular disease	205 (19.1)
Chronic lung disease	202 (18.9)
Chronic renal failure	304 (28.4)
Cancer	244 (22.8)
Diabetes	163 (15.2)
Charlson index, median (IQR)	6 (4–8)
Central venous catheter at isolation	414 (38.7)
Urinary catheter at isolation	562 (52.5)
Immunosuppressive therapy	209 (19.5)
Days of hospitalization, median (IQR)	25 (14–45)
KPC-*Kp* acquisition characteristics‡	
Severe infection	221 (20.6)
Mild infection	109 (10.2)
Colonization$_{sur}$	741 (69.2)
Median time from hospitalization to isolation of strain, d (IQR)‡	
Severe infection	12 (2–22)
Mild infection	11 (2–25)
Colonization$_{sur}$	6 (1–17)
Median time from strain isolation to discharge or death, d (IQR)‡	
Severe infection	18 (9–35)
Mild infection	20 (12–35)
Colonization$_{sur}$	13 (6–22)

*Values are no. (%) except as indicated. IQR, interquartile range; KPC-*Kp*, *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae*.
†Underlying conditions and devices are listed when present in ≥10% of patients.
‡Severe infection included bloodstream or lower respiratory tract infection plus septic shock from other sites; Mild infection included infections from other sites; and colonized$_{sur}$ patients were identified through surveillance protocols.

Epidemiology of KPC-*Kp*

During 2017, the estimated prevalence of KPC-Kp among hospitalized patients in the Lombardy region was 3.26 (95% CI 2.99–3.54) per 1,000 admissions. In the same region, the overall cumulative incidence of KPC-Kp infections was 1.00‰ (95% CI 0.86‰–1.16‰) and the incidence of acquired infections occurring >48 hours after hospital admission was 0.68‰ (95% CI 0.56‰–0.82‰). The proportion of patients infected at admission, considered imported infections, was ≈30% in most centers. We observed marked differences across centers even after standardization by age and ward of isolation, with values ranging from 1.62‰ (95% CI 1.07‰–2.18‰) in center A to 0.21‰ (95% CI 0.02‰–0.40‰) in center B (Appendix Figure 3).

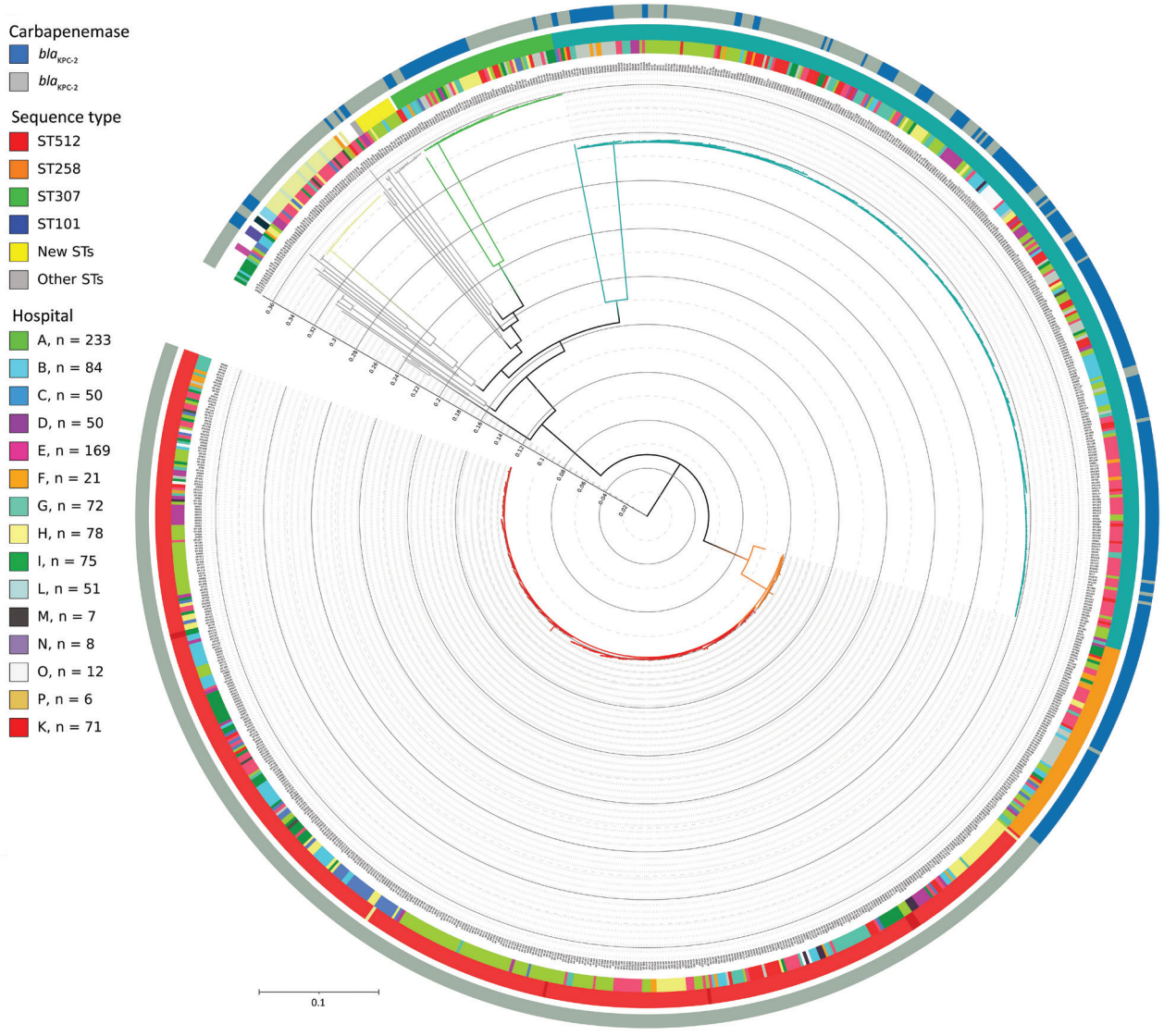

Figure 2. Phylogenetic tree of 989 *Klebsiella pneumoniae* genomes isolated at hospitals participating in the KPC-producing *K. pneumoniae* (KPC-*Kp*) study, Italy. The key shows the number of isolates included in the study provided by each center; 2 samples (1 from each from hospitals A and I) were excluded because the total quality of the assemblies was not sufficient to have high confidence in the SNPs called through all the genome (total coverage <30). Inner circle shows the KPC-*Kp* mechanism identified; middle circle shows hospitals from which strains were isolated; and outer circle shows identified STs. The whole genome core single-nucleotide polymorphisms (SNPs) were extracted from the 989 *K. pneumoniae* genome assemblies by using kSNP3.0 (https://sourceforge.net/projects/ksnp). Parametric maximum-likelihood estimation (general time-reversible plus gamma distribution plus invariable sites) analysis with 1,000 bootstrap estimates was used to infer the phylogeny. We used IQ-TREE (http://www.iqtree.org) to generate the tree and iTOL (https://itol.embl.de) to draw the tree. Major STs are represented by branch colors; ST512 and ST307 were the predominant STs. Major branches have bootstrap values >0.75 for branch support. Scale bar indicates nucleotide substitutions per site. KPC, *Klebsiella pneumoniae*–carbapenemase; ST, sequence type.

Patient Outcomes

In-hospital death from all causes was 34% (95% CI 29.2%–39.6%) among KPC-*Kp*–infected patients and 21% (95% CI 17.7%–27.6%) among colonized patients. No differences emerged when we stratified for carbapenem-resistance mechanisms and the most prevalent clones (Appendix Table 4).

Mortality hazards (considering the first 15 days after KPC-Kp isolation), were much higher for patients with severe infection than for colonized patients, even after controlling for center, time between hospitalization and isolation, age, ward of isolation, and Charlson index (adjusted HR [aHR] = 1.93, 95% CI 1.40–2.66) (Table 2). In contrast, no excess mortality hazard was noted for patients with mild infections (aHR = 0.75, 95% CI 0.42–1.34) compared with colonized patients."

When we analyzed the subgroup of patients with bloodstream infections, we found clinical manifestation of septic shock more than doubled the risk for death (HR = 2.71, 95% CI 1.46–5.02). We found comparable results when we excluded from the analysis 343 patients discharged alive before day 15 (data not shown).

Antimicrobial Drug Treatment

On the basis of susceptibility test results, we found that 54% (159/297) of patients infected with KPC-*Kp* received adequate empirical therapy (Appendix Table 5). Empirical treatment was most frequently adequate in patients with KPC-*Kp* colonization during the previous 12 months and in patients with severe infection (Appendix Table 5).

Fewer treatment delays (<4 days, which is considered the maximum acceptable waiting time to receive appropriate antimicrobial treatment) were reported for patients with severe KPC-*Kp* infection than patients with mild infections (Table 3). Patients reporting KPC-*Kp* colonization during the previous 12 months more frequently received prompt adequate therapy (p<0.001).

Among the 282 KPC-*Kp*–infected patients treated for their infections, 62 (22%) received an in vitro active drug plus carbapenem, but 29 (10%) patients received gentamicin, fosfomycin, or tigecycline monotherapy. The most common drug combination was colistin plus tigecycline plus carbapenem, which most frequently was administered to patients with severe infections. Ceftazidime/avibactam became available in Italy in February 2018, and 26/39 (66%) infected patients included after that date received it: 19/24 (79%) in the severe infection group and 7/15 (47%) in mild infection group (Appendix Table 6).

Discussion

This study provides a detailed picture of KPC-*Kp* burden in an endemic setting and shows that KPC-*Kp* poses a major challenge for Italy's healthcare system. We estimated that 1 of every 1,000 patients admitted to participating hospitals during 2017 had a positive KPC-*Kp* specimen during hospitalization, which is ≈10 times the estimated number of CRE infections in Europe (1.3/10,000 hospitalizations) (*7*). This high rate is at least partly compatible with the heterogeneity in the surveillance protocols adopted by hospitals. Another factor contributing to the high rate of KPC-*Kp* could be the older age of the patient population, most of whom were men >65 years of age. In 2017, the median age of the adult population in Lombardy was 50 years, but the median age for the 170,699 adult patients in our study was 66 years, and 27% were >77 years of age. Of note, the considerable proportion of imported KPC-*Kp* infections, ≈30%, for most centers, suggests that active surveillance might need to be

Table 2. In-hospital death within 15 days of KPC-*Kp* isolation in a cohort of infected patients and subgroup of patients with bloodstream infections, Italy*

KPC-*Kp* infections	No.	Died, no. (%)	HR (95% CI)†	p value	HR (95% CI)‡	p value
All patients	1,039	174 (16.7)	NA	NA	NA	NA
Severity of infection§						
Colonized	712	100 (14.0)	Referent	NA	Referent	NA
Mild	109	13 (11.9)	0.71 (0.40–1.27)	0.247	0.75 (0.42–1.34)	0.328
Severe	218	61 (28.0)	1.84 (1.34–2.54)	0.0002	1.93 (1.40–2.66)	<0.0001
Bloodstream infections	176	45 (25.6)	NA	NA	NA	NA
Septic shock at admission						
N	132	25 (18.9)	Referent	NA	Referent	NA
Y	44	20 (45.5)	2.72 (1.50–4.90)	0.0009	2.71 (1.46–5.02)	0.002

*All patients are stratified for severity of infection; the subgroup of patients with bloodstream infection is stratified for septic shock. KPC-*Kp, Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae*; NA, not applicable.
†Hazard ratio (HR) estimates are from multivariable Cox proportional hazard models, adjusting for center (random effect) and days elapsing from hospitalization to KPC-*Kp* isolation.
‡Multivariable Cox mixed effects model adjusting for center (random effect) and days elapsing from hospitalization to KPC-*Kp* isolation, age, Charlson Index, and whether or not isolates were collected when patient was in the intensive care unit.
§Patients discharged or deceased on the day of KPC-*Kp* isolation were excluded from analyses; 20 patients were discharged, 9 colonized patients died, and 3 colonized patients had severe infections.

Table 3. Association between delay in receiving adequate antimicrobial therapy after KPC-*Kp* isolation and selected patient characteristics, Italy*

Characteristics	Delay from KPC-*Kp* isolation to adequate antimicrobial therapy		χ^2 p value	p value†
	<4 d	≥4 d		
All	190 (63.9)	107 (36.0)	NA	NA
Age, median (IQR)	68.5 (62–78)	74 (63–81)	0.151	0.285
Charlson Index, median (IQR)	5.0 (4–8)	6.0 (4–8)	0.615	0.439
Intensive care unit admission				
Y	41 (63.1)	24 (36.9)	0.865	0.354
N	149 (64.2)	83 (35.8)		
Previous KPC-*Kp* colonization during the current hospitalization				
Y	46 (74.2)	16 (25.8)	0.060	0.118
N	144 (61.3)	91 (38.7)		
KPC-*Kp* colonization in the previous 12 mo				
Y	104 (77.0)	31 (23.0)	<0.001	<0.001
N	86 (53.2)	75 (46.8)		
Hospitalization in the previous 12 mo				
Y	149 (64.5)	82 (35.5)	0.832	0.779
N	41 (63.1)	24 (36.9)		
Antimicrobial therapy in the 30 d before hospitalization				
Y	145 (64.0)	84 (36.0)	0.564	0.627
N	45 (67.2)	22 (32.8)		
Major surgery‡				
Y	48 (53.9)	41 (46.1)	0.018	0.008
N	142 (74.7)	66 (31.7)		
KPC-*Kp* infection severity§				
Severe	139 (71.5)	55 (28.3)	0.0002	<0.001
Mild	52 (50.0)	52 (50.0)		

*Values are no. (%) except as indicated. Delay determined according to infected patients' resistance profiles; 33 patients were excluded: 17 had follow-up <3 days after isolation and 16 had no data on empirical therapies. IQR, interquartile range; KPC-*Kp*, *Klebsiella pneumoniae*-carbapenemase producing *Klebsiella pneumoniae*; NA, not applicable.
†Obtained from multivariable mixed logistic model adjusted by center, as random effect; age; and type of KPC-*Kp* infection, when appropriate.
‡Major surgery includes *any invasive operative procedure in which a more extensive resection is performed, including a body cavity is entered, organs are removed, or normal anatomy is altered*.
§Severe infection included bloodstream or lower respiratory tract infection plus septic shock from other sites; Mild infection included infections from other sites; and colonized patients were identified through surveillance protocols.

extended to post-acute care, long-term care, or rehabilitation facilities to control the spread of KPC-*Kp*. As highlighted by a recent report from the European Centre for Disease Prevention and Control (*16*), standardized actions for CRE containment in Italy must be driven by comprehensive coordinated responses implemented nationally rather than current practice of delegating responsibilities to the regional or hospital level.

In our setting, the KPC-*Kp* epidemic appears to be driven by the expansion of 3 major *K. pneumoniae* clonal lineages, specifically ST307, ST101, and ST258/ST512. Those epidemic clones have been associated with outbreaks and are reported to have an increased capacity to acquire drug resistance (*17*–*19*). Clone ST512 was widely distributed across the centers in our study, confirming its spread in Italy (*20*). We noted clone ST307 in smaller, scattered clusters but did not note differences in infection severity or death between clones.

We examined the KPC-*Kp*–associated mortality rate and noted it was highest among patients with severe infections, particularly bloodstream infections with septic shock, which is consistent with previous research (*21*–*25*). We found no excess risk for death among patients with mild infection. KPC-*Kp* often is found in vulnerable hospital populations at high risk for illness and death (*21*,*26*). To estimate the effect of KPC-*Kp* infection on hospital mortality rates, we compared patients with severe and mild infections with colonized patients. Colonized patients who did not have infectious events during hospitalization represented the best available control group because they were hospitalized in the same hospitals at the same time as KPC-*Kp* infected cases and are known to have similar clinical characteristics and underlying conditions (*27*).

Regarding therapeutic approaches, we found the initial empirical selection of antimicrobial drug treatment was more frequently adequate in patients with a known previous KPC-*Kp* colonization. This result is in line with other published studies reporting that for patients with no history of previous colonization, adequate antimicrobial treatment can only be started once the susceptibility profile has been received, and this delay might lead to unfavorable outcomes (*28*–*31*). Thus, in geographic regions with high CRE prevalence, extending rectal swab

specimen surveillance to a broader at-risk hospital population is crucial to reduce time to adequate antimicrobial therapy and, ultimately, to improve patients' outcomes. As previously observed (*4*,*29*), a combination of ≥2 active agents have been prescribed predominantly in patients with severe infections and at higher risk for death. Of note, we observed a substantial use of colistin despite its unknown efficacy and poor safety profile (mainly related to renal failure), as documented in other studies (*32*–*34*). In addition, ceftazidime/avibactam use has increased since 2018, when it became available for routine clinical use in Italy. However, the use of ceftazidime/avibactam in nonbacteremic infections should be discouraged to reduce chances of acquired in vitro resistance (*35*–*37*). The wide variety of therapeutic regimens, >30 combinations reported in our centers, confirms the need for multicenter randomized trials to identify the most effective combination and dosage of antimicrobial agents.

The major strengths of our study are the size of the sample and the representation of KPC-*Kp* patients included with homogeneous methodology through an independent network of Lombardy hospitals of different size. The results reveal the multifaceted reality of KPC-*Kp* infection in clinical settings.

The first limitation of our study is that we focused on the most clinically relevant episode for each patient. Therefore, patients who had a colonization followed by an infection were considered and classified according to this second more severe event only. However, in our setting, this subgroup included only 8% of the colonized patients. Second, we limited our attention to KPC-*Kp* strains, ignoring *E. coli* and other carbapenemase, such as oxacillinase 48-like and New Delhi metallo-β-lactamase. Nevertheless, the estimated ratio of *K. pneumoniae* to *E. coli* was 11:1 in Italy (*16*), and KPC is the only endemic mechanism demonstrating carbapenem resistance (*9*). Third, despite the inclusion of a large number of infected patients, the multitude of treatment patterns prevented reliable exploration of effects of treatment on clinical outcomes, but the description of this heterogeneity remains one of the findings of this study. Finally, we focused on overall rather than disease-specific mortality rates because we aimed to give a global picture of KPC-*Kp* burden in the Lombardy region. Cause-specific mortality analysis would have required detailed information on the procedures performed before the events occurring during hospitalization, which was beyond the scope of this study.

In conclusion, our study describes KPC-*Kp* in a single region of Italy where KPC-*Kp* has been endemic since 2013. The KPC-*Kp* epidemic appears to be driven by the expansion of only 3 major clonal lineages. Therefore, the wide heterogeneity in the proportion and incidence of KPC-*Kp* infections are presumably largely influenced by surveillance protocols and hospital policies. Consequently, to reverse this trend, Italy needs a strengthened collaborative surveillance system that includes regional plans and strong, centrally coordinated activities at the national level. Furthermore, the wide range of treatments adopted by healthcare facilities in this study highlights the urgent need to accompany the surveillance system with a concerted, aggressive, and prompt antimicrobial stewardship plan.

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M.R., L.C., and A.G. designed the study and obtained funding. M.R. supervised the study conduction. L.C. supervised the statistical analysis. T.I. was responsible for conduction of data collection. D.C. was responsible for preparation and storage of all samples. P.M., S.B., C.A., R.M., P.A.G., C.D.C., S.P., S.G.R., P.B., P. Bonfanti, E.V.H., M.P., G.G., and C.C. collected data. F.G. and D.M.C. performed and analyzed the whole genome sequencing of all samples collected. L.C., I.S., and A.D.A. performed statistical data analysis. The paper was written by M.R., L.C., D.M.C., and A.G. and critically revised by G.N., M.C.R., and A.B. All authors reviewed and approved the final version of the manuscript before submission. The KPC-*Kp* Study Group contributed substantially to design the data collection form, to reach a shared infection criteria definition, and to enroll all the patients included in the study.

The de-identified patient data used for the results reported in this article, including data in text, tables, figures, and appendices, will be shared along with the study protocol. Data will be available from 3 months to 5 years after article publication. Data will be available to researchers who provide a methodologically sound proposal to achieve their aims. Proposals should be addressed to marianna.rossi@asst-monza.it. To gain access, data applicants will need to sign a data access agreement.

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Characteristics and Clinical Implications of Carbapenemase-Producing *Klebsiella pneumoniae* Colonization and Infection, Italy

Appendix

Extended Methods

Study Design, Setting and Patients

Center Characteristics

The study was conducted in 15 healthcare centers in Lombardy, the most populous region in Italy with 10,000,000 inhabitants (16.5% of the Italian population). These represent all regional centers with both a microbiology and an infectious diseases unit. The centers are distributed across the regional territory, providing extensive coverage. Among 15 participating centers, 8 are large tertiary care institutions with >25,000 admissions per year, offering a full range of clinical and surgical services (Appendix Table 1). All hospitals had a general medicine unit, various branches of surgery and ≥1 intensive care unit (ICU). Our study setting included a considerable proportion of immunosuppressed patients: 13 centers had a hematology unit and 8 a solid organ transplantation unit. In addition, 4 centers had a geriatric ward (Appendix Table 1).

In most centers, patients were empirically treated with currently standard doses of drugs with known gram-negative activity, either alone or with other antimicrobial drugs. Because the study is observational, empirical treatment regimens were at the discretion of the treating physician, in most cases in agreement with the infectious disease consultant, without the aid of a predefined protocol. Moreover, in some centers the policy of empirical therapy was based on 2 factors: the severity of the infection and the presence of risk factors for carbapenem-resistant *Enterobacteriaceae* (CRE).

Patient Infection Classification

When a single patient had multiple KPC-*Kp* infections, the most clinically relevant infection was considered in the analysis. For example, if a patient experienced a urinary tract infection followed by a bloodstream infection, only the bloodstream infection and related isolate were considered.

Personal Data Pseudonymization Process

The patient's name and date of birth were pseudonymized automatically with the generation of a patient identification number saved in the database. Only center staff could visualize personal data saved in a separate file.

Definition of Acquired Infections Adopted in All Centers

Patients with acquired infections were those with an infection arising >48 hours of admission (*1*). For infectious events, clinical presentation of septic shock, defined as sepsis with organ dysfunction and persistent hypotension despite volume replacement (*2*); chronic renal failure; antimicrobial treatment including empirical treatment, which was considered adequate when it included $\geq$1 drug with in vitro activity against the KPC-*Kp* isolate; and post-antibiogram treatment regimen were recorded.

Microbiology and Genomic Analysis of Strains

During the study period, the laboratory of each of the 15 participating centers collected consecutive, nonreplicate, clinical isolates of KPC-*Kp* from any site of infection or colonization of patients enrolled in the study.

Isolates were identified with the Vitek 2 system (bioMérieux, https://www.biomerieux.com) or matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry by MALDI Biotyper (Bruker Daltonics GmbH, https://www.bruker.com) or Vitek-MS (bioMérieux). Each hospital conducted antimicrobial susceptibility testing via automated systems and according to standard protocols. Eleven centers used the Vitek 2 system (bioMérieux); 3 centers used Phoenix (Becton Dickinson, https://www.bd.com); 1 center used MicroScan Walkaway (Siemens, https://www.siemens.com). One center confirmed all MICs by broth microdilution method, whereas others confirmed only selected antimicrobial MICs by broth microdilution or E-test. Results were interpreted in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (*3*).

All centers performed phenotypic detection of carbapenemase types by combination disk/MHT according to EUCAST guidelines. Only 1 center further analyzed the isolates for the presence of KPC by using an immunochromatographic test: KPC K-SeT test (Coris Bioconcept, https://www.corisbio.com). Genetic detection of carbapenemases was performed in only 3 centers by using the Xpert Carba-R assay (Cepheid, http://www.cepheid.com).

Bacterial isolates were subcultured in blood agar medium (Becton Dickinson) and incubated overnight at 37°C. DNA was extracted from a liquid suspension of the isolated colonies by using the Maxwell 16 Cell DNA Purification Kit SEV (Promega, https://www.promega.com) in combination with a Maxwell 16 Instrument (Promega) for automated isolation of genomic DNA. All strains were sequenced with the Illumina NextSeq500 platform (Illumina Inc., htpps://illumina.com), with a paired-end run of 2 by 150 bp, after Nextera XT (Illumina) paired-end library preparation.

Sequencing reads were assembled using SPAdes Genome version 3.13 (http://cab.spbu.ru/software/spades) with accurate de novo settings (*4*). The assembled contigs were evaluated with an automated bioinformatic pipeline for AMR gene detection (ResFinder v.3.0, https://cge.cbs.dtu.dk/services/ResFinder), available at the Center for Genomic Epidemiology (*5*). Phylogenic analysis was based on core-genome single nucleotide polymorphism (SNP) sequences (47,895 SNPs) obtained from the analysis of 989 *Klebsiella pneumoniae* genomes using kSNP3.0 (https://sourceforge.net/projects/ksnp). Parametric maximum-likelihood estimation (model: GTR+G+I) analysis with 1,000 bootstrap estimates was used to infer the phylogeny; IQ-TREE (http://www.iqtree.org) was used to generate the tree; iTOL (https://itol.embl.de) was used for graphic representation of the tree (Figure 2). Major branches have bootstrap values >0.75 for branch support (*6*).

Statistical Analysis

Categorical variables are presented as frequency and proportion (%) and continuous variables as median, lower and upper quartiles (Q1–Q3). The most common reasons for nonenrollment were a delay in communications between the microbiologist and physician responsible for patient enrollment, patient transfer to other facilities before being enrolled, or both.

To estimate the various indicators describing KPC-*Kp* epidemiology that emerged from the implemented surveillance system, without modifying or interfering with current clinical practices and hospital policies, we retrieved information on all hospitalized patients, irrespective of KPC-*Kp* surveillance, available from the hospitals' administrative data repositories for the year 2017. Centers were considered for the calculation of KPC-*Kp* prevalence and cumulative incidence of infection only if they provided the administrative database of all in-patient admissions and enrolled ≥85% of all adult patients for whom a positive KPC-*Kp* isolate was collected, as recorded in the microbiology laboratory database. Among 15 participating centers, 8 were included in our calculations. We excluded 6 centers because their period of enrollment did not cover all 12 months of 2017 (Appendix Figure 1), and we excluded 1 center that had retrieved data for <85% of the KPC-*Kp* patients registered in the microbiology laboratory database during 2017 (Figure 1). We then merged this database with information on KPC-*Kp* patients available in the study cohort database.

KPC-*Kp* patients were classified, according to the most clinically relevant KPC-*Kp* event, into 3 mutually-exclusive groups: never infected-colonized patients (Ncol), patients infected within ≤48 hours since hospital admission (Nadm), and those with an infection occurring later during hospitalization (i.e., hospital-acquired infections, Nstay). The various measures were calculated as follow: the prevalence of KPC-*Kp* in hospitalized patients in the region of Lombardy during 2017 (Ptot = 1,000*(Ncol+Nadm+Nstay)/Ntot), the prevalence of KPC-*Kp* non-infected colonized patients in the same population (Pcol = 1000*Ncol/Ntot), the cumulative incidence of acquired infections among hospitalized patients in the region of Lombardy in 2017 (Pinf = 1000*(Nadm+Nstay)/Ntot), and the cumulative incidence of acquired infections occurring >48 hours of hospital admission among hospitalized patients in the region of Lombardy in 2017 (CI = 1,000* Nstay/Ntot). Prevalence/incidence were reported as crude rates and standardized by age (lower or higher than 66 years, the median of the 170,699 patients admitted) and ward of isolation (ICU, infectious diseases, surgery, oncology/hematology, and other medical wards), according to a direct method ($_{std}$P). The standard population was the overall adult population of patients admitted in 2017 in these 8 centers, excluding day admissions and pediatric admissions. We calculated 95% CI by using Poisson distribution (Appendix Figure 3).

To study the role of KPC-*Kp* infection severity on 15-day mortality rates in KPC-*Kp* patients, we considered the exposure variable of KPC-*Kp* infections as severe, mild, or colonized. We conducted a survival analysis in which the time of KPC-*Kp* isolation was taken as time of origin (i.e., t = 0), and the event was hospital death occurring ≤15 days of KPC-*Kp* isolation, thus we censored hospital stays at 15 days. Colonized patients were selected as the reference category since they represented the best available control group because they were hospitalized during the same time and at the same locations in which the KPC-*Kp*-infected cases arose, and had comparable clinical characteristics (Appendix Table 2). Multivariable Cox proportional hazard frailty models on 15-day hospital mortality rates were used to estimate both crude and adjusted hazard ratios (HRs). To select covariates in the Cox proportional hazard mixed models, we constructed several Cox proportional hazard mixed models (i.e., center was entered as random effect) to identify factors independently associated with 15-day hospital mortality rates. In particular, we included the following: previous colonization (yes/no), previous hospitalization (yes/no); isolation ward ICU (yes/no); antimicrobial therapy in the 30 days before hospitalization (yes/no); major surgery in the 30 days before isolation (yes/no); Charlson Index; underlying conditions, including congestive heart failure (yes/no), peripheral vascular disease (yes/no), cerebrovascular disease (yes/no), chronic lung disease (yes/no), chronic renal failure (yes/no), cancer (yes/no), or diabetes (yes/no); central venous catheter at isolation, urinary catheter at isolation, immunosuppressive therapy (yes/no); carbapenem-resistance mechanism (KPC3 vs KPC2); and major clones ST512 (yes/no) or ST307 (yes/no). Only the number of days between admission and KPC-*Kp* isolation were considered for KPC-*Kp* patients as a covariate in the first models. A center-specific random intercept was also included, to adjust for potential center-specific effects. Hospital stays were censored at 15 days. Thus, factors were entered into the adjusted models on the basis of their univariate relation to outcome ($p<0.20$) for differences in confounding factors between types of KPC-*Kp* patients. All factors were biologically plausible with a sound scientific rationale. However, if the Pearson or Spearman correlation coefficient (according to variables distribution), was >0.30, the variable with the lower p value was retained in the model (for example, when central venous catheter was considered, ICU was excluded as the ward of isolation), or if the Charlson Index was included in the multivariable model, components of this score (such as renal failure) were not included separately. The proportional hazards (PH) assumption was checked by graphical diagnostics based on the scaled Schoenfeld

residuals, entering time-dependent covariates for each covariate, included 1 by 1 in the model, and verifying estimated coefficients with $p<0.05$.

When the main targeted therapeutic regimens were compared between the severe and mild infection groups, we used χ^2 and Fisher exact tests to compare categorical variables and *t*-test or Mann-Whitney U test to compare continuous variables. We performed all analyses by using SAS 9.4 software (SAS Institute, Inc., https://www.sas.com) and considered $p\leq0.05$ statistically significant.

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Appendix Table 1. Description of the 15 study centers and their surveillance protocols (KPC-*Kp* study network)

Center characteristics				Surveillance protocols: Unit, rectal swab or other cultures done weekly									Risk factors†: Previous		
Center; no. adm/y	Ter	Teach	No. ICU beds	ICU	TP	OH	Surg	ID	GM	GE	Neph	Ger	Hospitalization	Colonization	Di
G; 51,000	Y	Y	32	Y	N	Y	N	N	N	NA	NA	NA	N	N	N
I; 44,900	Y	Y	24	Y	N	Y	Pancreatic	NA	N	N	N	NA	Y	Y	Y
N; 41,292	N	Y	26	Y	N	Y	N	N	N	N	N	NA	N	N	N
H; 40,000	Y	Y	32	Y	N	N	N	N	N	N	N	N	Y	Y	N
B; 37,000	Y	Y	76	Y	Y	Y	N	N	Y	Y	NA	NA	Y	Y	N
K; 34,908	Y	Y	21	Y	N	N	N	NA	N	N	NA	N	N	Y	Y
E; 31,297	Y	Y	42	Y	Y	N	Abd/hep	N	N	N	NA	NA	Y	Y	N
A; 27,600	Y	Y	24	Y	NA	Y	N	N	N	N	N	N	Y	Y	Y
D; 27,000	Y	N	30	Y	NA	N	N	N	N	NA	N	NA	Y	Y	Y
M; 21,480	N	N	8	Y	NA	Y	N	N	N	NA	N	NA	Y	Y	N
O; 19,225	Y	Y	17	Y	N	N	N	Y	N	NA	N	NA	Y	Y	Y
L; 18,287	Y	Y	6	Y	NA	NA	N	N	N	N	N	NA	N	N	Y
C; 18,167	N	N	8	Y	NA	Y	Urology	Y	N	N	Y	NA	N	Y	Y
P; 18,000	N	N	7	Y	NA	NA	N	N	N	NA	N	Y	Y	Y	Y
F; 15,869	Y	Y	14	Y	NA	NA	Abd	Y	Y	N	Y	NA	N	N	N

*Abd, abdominal; adm, admissions; Di, dialysis; GE, gastroenterology; Ger, gerontology; GM, general medical; hep, hepatic; ICU, intensive care unit; ID, infectious diseases; NA, not applicable; Neph, nephrology; OH, onco-hematology; Surg, surgical; Teach, teaching; Ter, tertiary; TP, solid organ transplant.
†Risk factors considered in active surveillance at admission.

Appendix Table 2. Characteristics and severity of KPC-*Kp* among patient enrolled from 15 healthcare centers during 2016–2018, Italy*

Characteristics	Severity of KPC-*Kp* infection: Severe	Mild	Colonized
All	221 (100)	109 (100)	741 (100)
Sex			
M	149 (67.4)	71 (65.1)	474 (64.0)
F	72 (32.5)	38 (34.9)	267 (36.0)
Median age (IQR)	70 (60–78)	75 (64–82)	72 (61–80)
Healthcare exposures before KPC-*Kp* onset			
Previous KPC-*Kp* colonization in the past 12 mo.	102 (46.2)	46 (42.2)	185 (25.0)
Previous hospitalization in the past 12 mo.	168 (76.0)	85 (78.0)	612 (82.6)
Antimicrobial therapy in the 30 d before hospitalization	166 (75.1)	85 (78.0)	531 (71.7)
Major surgery in the past 30 d	68 (30.8)	32 (29.4)	162 (21.9)
Underlying conditions†			
All underlying conditions	206 (93.2)	103 (94.5)	680 (91.8)
Congestive heart failure	35 (15.8)	20 (18.4)	137 (18.5)
Peripheral vascular disease	47 (21.3)	21 (19.3)	129 (17.4)
Cerebrovascular disease	35 (15.8)	19 (17.4)	151 (20.4)
Chronic lung disease	30 (13.6)	24 (22.0)	148 (20.0)
Chronic renal failure	57 (25.8)	34 (31.2)	213 (28.7)
Cancer	54 (24.4)	35 (32.1)	155 (20.9)
Diabetes	25 (11.3)	24 (22.0)	114 (15.4)
Any combination of CHF, PVD, or CRF	90 (40.7)	46 (42.2)	321 (43.3)
Other markers of the severity of underlying illness			
Median Charlson Index (IQR)	5.0 (3.0–7.0)	6.0 (4.0–9.0)	6.0 (4.0–8.0)
Central venous catheter at isolation	118 (53.4)	39 (35.8)	257 (34.7)
Urinary catheter at isolation	120 (54.3)	60 (55.1)	382 (51.5)
Immunosuppressive therapy	55 (24.9)	20 (18.4)	134 (18.1)

*Data are no. (%) except where otherwise noted. Severe infection included bloodstream or lower respiratory tract infection plus septic shock from other sites; Mild infection included infections from other sites; and colonizedsur patients were identified through surveillance protocols. CHF, congestive heart failure; CRF, chronic renal failure; KPC-*Kp*, *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae*; PVD, peripheral vascular disease.
†Underlying conditions were included when present in ≥10% of all patients.

Appendix Table 3. Number of isolates according to sequence type and *Klebsiella pneumoniae* carbapenemase-producing variants reported for 15 healthcare centers participating in KPC-*Kp* surveillance, Italy*

Center code; total no. isolates	No. KPC variants		Sequence types, no.				
	KPC-2	KPC-3	ST101	ST258	ST307	ST512	Others
All	313	676	57	71	326	441	94
A; n = 234	58	176	6	11	79	118	20
B; n = 84	36	48	3	7	26	43	5
C; n = 50	6	44	3	3	2	34	8
D; n = 50	18	32	1	2	20	22	5
E; n = 169	80	89	6	15	73	49	26
F; n = 21	9	12	0	4	8	8	1
G; n = 72	10	62	5	4	25	34	4
H; n = 78	13	65	11	2	10	48	7
I; n = 76	14	62	7	6	8	44	11
K; n = 71	21	50	6	3	41	19	2
L; n = 51	35	16	8	12	26	5	0
M; n = 7	1	6	0	0	0	3	4
N; n = 8	1	7	0	0	1	7	0
O; n = 12	7	5	0	0	7	5	0
P; n = 6	4	2	1	2	0	2	1

*KPC-*Kp*, *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae*; ST, sequence type

Appendix Table 4. Overall in-hospital mortality rates among patients infected or colonized by KPC-*Kp* according to selected characteristics*

Characteristics	KPC-*Kp* colonized, n = 741		KPC-*Kp* infected, n = 330	
	No. (%)	**95% CI**	**No. (%)**	**95% CI**
Overall rates	152 (20.5)	17.7%–27.6%	113 (34.2)	29.2%–39.6%
Median days from hospitalization to strain isolation (IQR)	7 (1.0–17.5)	NA	29 (15.0–47.0)	NA
Median days from strain isolation to death (IQR)	8 (3.0–17.5)	NA	12 (5.0–18.0)	NA
Intensive care unit admission				
Y	70/192 (36.5)	29.6%–43.7%	39/75 (52.0)	40.2%–63.7%
N	82/549 (14.9)	12.1%–18.2%	74/255 (29.0)	23.5%–35.0%
Previous KPC-*Kp* colonization	17/185 (9.2)	5.4%–14.3%	55/148 (37.2)	29.4%–45.5%
Previous hospitalization	118/612 (19.3)	16.2%–22.6%	85/253 (33.6)	27.8%–39.8%
Antimicrobial therapy in the 30 d before hospitalization	112/531 (21.1)	17.7%–24.8%	89/251 (35.5)	29.5%–41.7%
Major surgery	36/162 (22.2)	16.1%–29.4%	40/100 (40.0)	30.3%–50.3%
Underlying conditions	145/680 (21.3)	18.3%–24.6%	109/309 (35.3)	29.9%–40.9%
Carbapenem-resistance mechanism†				
KPC-2	35/219 (16.0)	11.4%–21.5%	36/94 (38.3)	28.5%–48.9%
KPC-3	96/440 (21.8)	18.0%–26.0%	77/236 (32.6)	26.7%–39.0%
Most frequent clones				
ST101	10/40 (25.0)	12.7%–41.2%	7/17 (41.2)	18.4%–67.1%
ST258	8/49 (16.3)	7.3%–29.7%	7/22 (31.8)	13.9%–54.9%
ST307	42/220 (19.1)	14.1%–24.9%	34/106 (32.1)	23.3%–41.8%
ST512	60/283 (21.2)	16.6%–26.4%	56/158 (35.4)	28.0%–43.4%
Infection severity‡				
Mild	NA	NA	23/109 (21.1)	13.9%–30.0%
Severe	NA	NA	90/221 (40.7)	34.2%–47.5%
Bloodstream infections	NA	NA	65/179 (36.3)	25.1%–39.2%
With septic-shock	NA	NA	24/45 (53.3)	37.9%–68.3%
Without septic-shock	NA	NA	41/134 (30.6)	22.9%–39.1%

*Among colonized patients, 82 had no available data on genotyping. KPC-*Kp*, *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae*; ST, sequence type.

‡Severe infection included bloodstream or lower respiratory tract infection plus septic shock from other sites; Mild infection included infections from other sites; and colonizedsur patients were identified through surveillance protocols.

Appendix Table 5. Relations between empirical antimicrobial drug appropriateness and selected characteristics among patients with KPC-*Kp* infections, Italy*

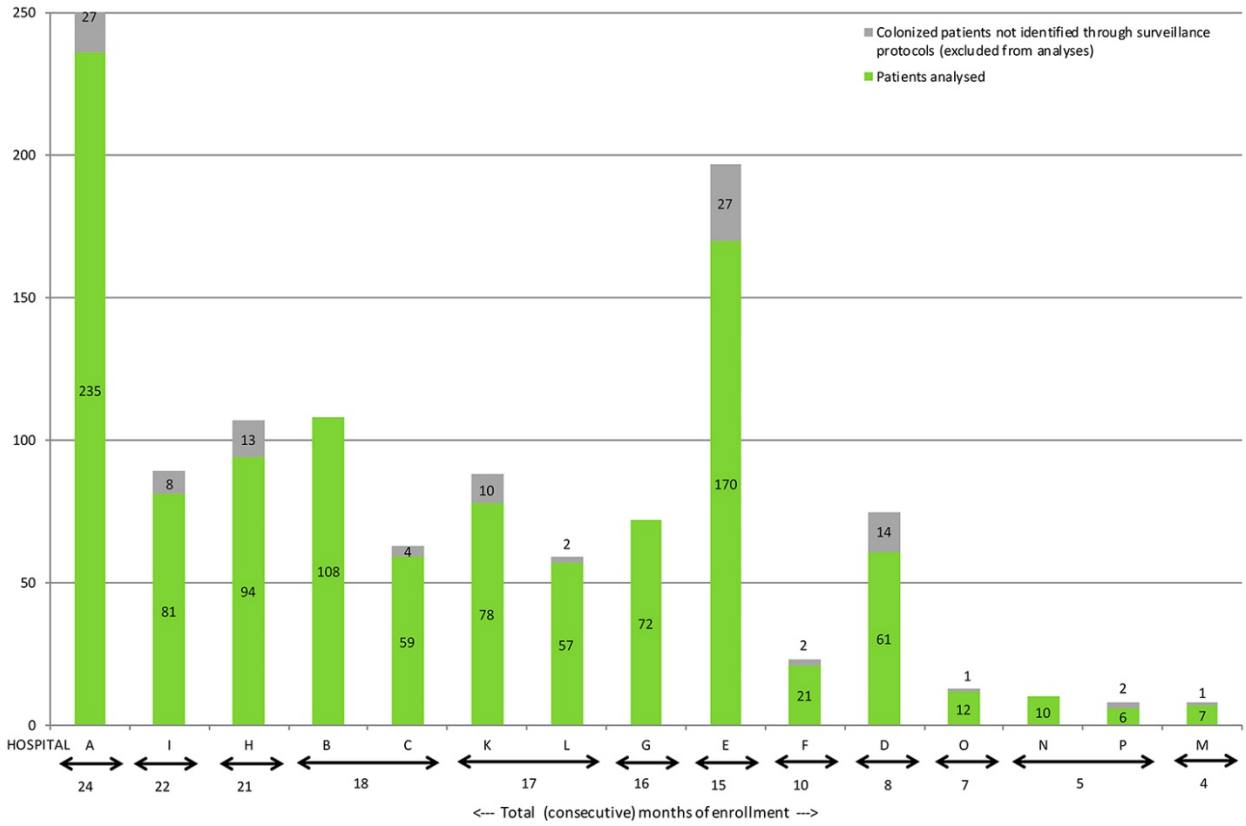
Characteristics	Empirical therapy		χ^2 p value	p value†
	Inadequate	Adequate		
All	138 (100)	159 (100)	NA	NA
Median age (IQR)	74 (60–80)	68 (73–78)	0.260	0.757
Charlson Index	6 (4–8)	5 (4–8)	0.365	0.984
Intensive care unit admission				
Y	31 (47.7)	34 (52.3)	0.822	0.320
Previous KPC-*Kp* colonization during the current hospitalization				
Y	21 (33.9)	41 (66.1)	0.025	0.056
N	117 (49.8)	118 (50.2)	Referent	Referent
Previous KPC-*Kp* colonization in the past 12 mo.				
Y	44 (32.6)	91 (67.4)	<0.001	<0.001
N	93 (57.8)	68 (42.2)	Referent	Referent
Previous hospitalization in the past 12 mo.				
Y	107 (46.3)	124 (53.7)	0.981	0.943
N	30 (46.2)	35 (53.8)	Referent	Referent
Antimicrobial therapy in the 30 d before hospitalization				
Y	112 (48.9)	117 (51.1)	0.094	0.062
N	25 (37.3)	42 (62.7)	Referent	Referent
Major surgery				
Y	50 (56.2)	39 (43.8)	0.028	0.016
N	88 (42.3)	120 (57.7)	Referent	Referent
KPC-*Kp* infection severity‡				
Severe	74 (38.1)	120 (61.9)	<0.001	0.0003
Mild	64 (62.1)	39 (37.9)	Referent	Referent

*Antimicrobial drug appropriateness determined according to the bacteria resistance profile and selected characteristics. Thirty-three patients were excluded: 17 had follow-up <3 d, 16 had no data on empirical therapies. Values represent no. (%) except where otherwise indicated. KPC-*Kp*, *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae*.
†Estimates from multivariable mixed logistic model adjusted by center (as random effect), age and type of KPC-*Kp* infection
‡Severe infection included bloodstream or lower respiratory tract infection plus septic shock from other sites; mild infection included infections from other sites.

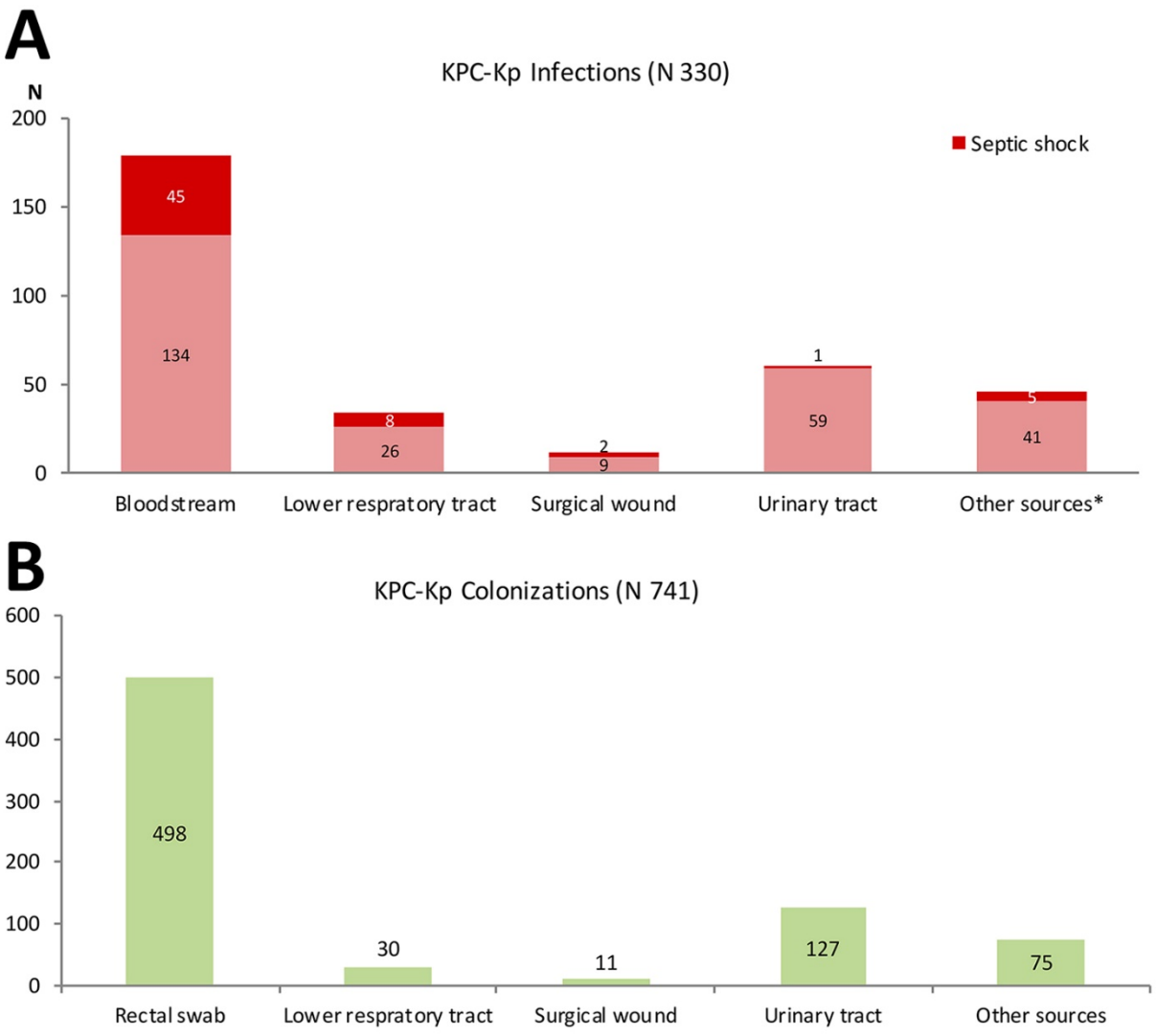
Appendix Table 6. Main targeted therapeutic regimens according to the severity of KPC-*Kp* infection, 2016–2018, Italy*

Therapy regimens	All	Severe	Mild	p value†
All infections	297 (100)	194 (100)	104 (100)	NA
Targeted therapy				
All therapies‡	282 (94.9)	182 (86.4)	100 (94.9)	NA
Double carbapenem	69 (24.5)	49 (26.9)	20 (20.0)	0.196
Colistin + tigecycline + carbapenem	53 (18.8)	45 (24.7)	8 (8.0)	0.001
Gentamicin + tigecycline + carbapenem	15 (5.3)	11 (6.0)	4 (4.0)	0.585
Colistin + carbapenem	29 (10.3)	19 (10.4)	10 (10.0)	0.907
Gentamicin + carbapenem	14 (5.0)	4 (2.2)	10 (10.0)	0.007
Fosfomycin + carbapenem	10 (3.5)	5 (2.8)	5 (5.0)	0.333
Tigecycline + carbapenem	9 (3.2)	7 (3.9)	2 (2.0)	0.499
Gentamicin monotherapy	10 (3.5)	1 (0.5)	9 (9.0)	0.001
Fosfomycin monotherapy	10 (3.5)	2 (1.1)	8 (8.0)	0.005
Tigecycline monotherapy	9 (3.2)	5 (2.7)	4 (4.0)	0.725
CAZ-AVI combined with others, n = 39§	26 (66.7)	19/24 (79.2)	7/15 (46.7)	0.033

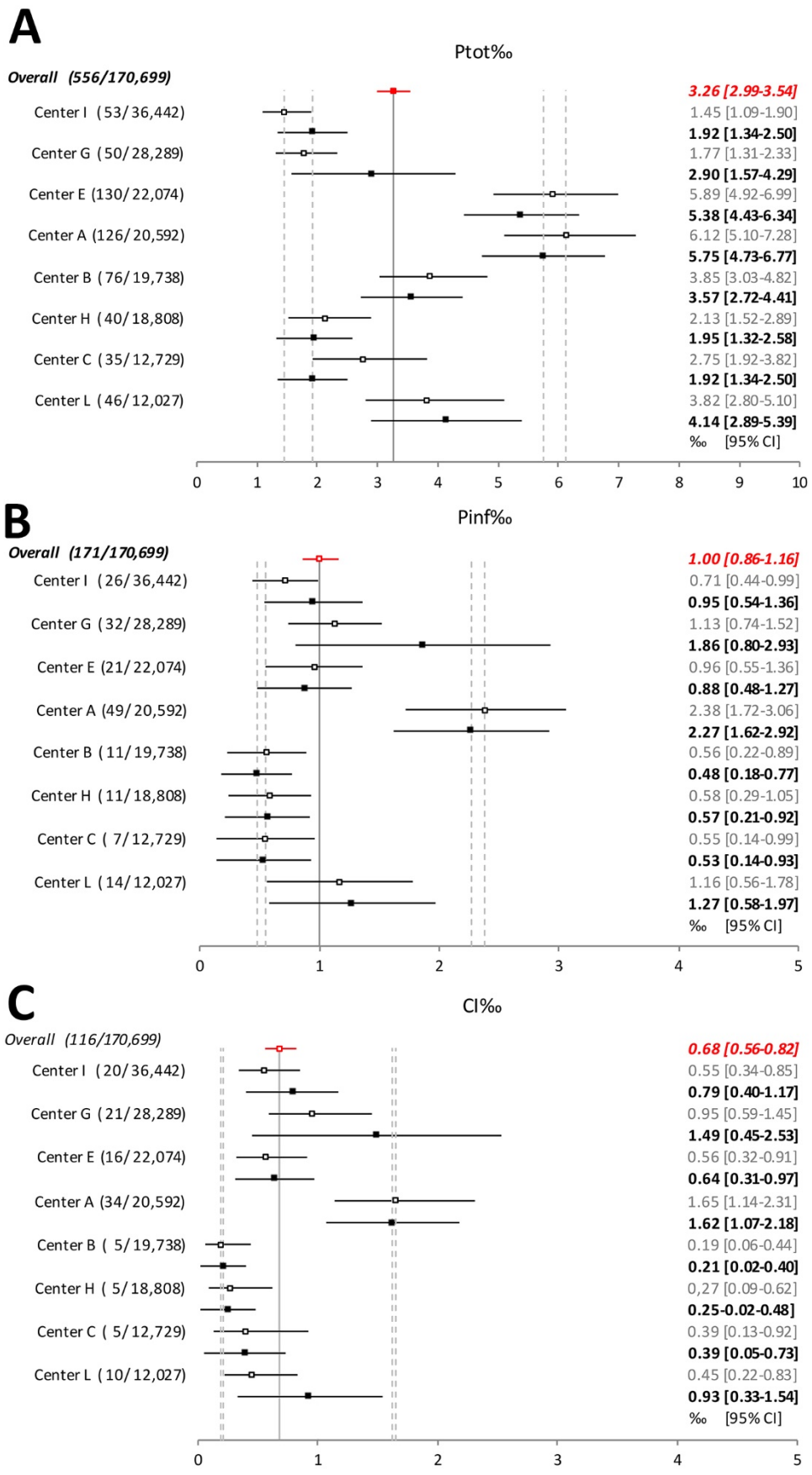
*CAZ-AVI, ceftazidime-avibactam; KPC-*Kp*, *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae*.
†p values refer to χ^2 or Fisher exact test, when appropriate.
‡Fifteen patients had missing details of therapies.
§CAZ-AVI became available in February 2018; only 39 of 295 patients who received a targeted therapy were enrolled after that date: 24 had bloodstream or lower respiratory tract infections and 15 had infections from other sites. An additional 2 patients received CAZ-AVI before February 2018 for compassionate use. All patient infections had susceptibility to CAZ-AVI.



Appendix Figure 1. Number of patients and months of recruitment among participating hospitals in a study of *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae*, Italy. Centers A–P are shown in decreasing order of the number of months of consecutive participation.



Appendix Figure 2. Source of isolation for the *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* (KPC-*Kp*) infection and colonization, Italy. Isolations sources for 330 patients with KPC-*Kp* infection (A) and 741 colonized patients (B). *Other sources include 13 from pus and 33 from other sources, reported for <4 patients.


A
Ptot‰
Overall (556/170,699)
3.26 [2.99-3.54]
Center I (53/36,442)
1.45 [1.09-1.90]
Center G (50/28,289)
1.92 [1.34-2.50]
Center E (130/22,074)
1.77 [1.31-2.33]
Center A (126/20,592)
2.90 [1.57-4.29]
Center B (76/19,738)
5.89 [4.92-6.99]
Center H (40/18,808)
5.38 [4.43-6.34]
Center C (35/12,729)
6.12 [5.10-7.28]
Center L (46/12,027)
5.75 [4.73-6.77]
3.85 [3.03-4.82]
3.57 [2.72-4.41]
2.13 [1.52-2.89]
1.95 [1.32-2.58]
2.75 [1.92-3.82]
1.92 [1.34-2.50]
3.82 [2.80-5.10]
4.14 [2.89-5.39]
‰ [95% CI]
0 1 2 3 4 5 6 7 8 9 10
B
Pinf‰
Overall (171/170,699)
1.00 [0.86-1.16]
Center I (26/36,442)
0.71 [0.44-0.99]
Center G (32/28,289)
0.95 [0.54-1.36]
Center E (21/22,074)
1.13 [0.74-1.52]
Center A (49/20,592)
1.86 [0.80-2.93]
Center B (11/19,738)
0.96 [0.55-1.36]
Center H (11/18,808)
0.88 [0.48-1.27]
Center C (7/12,729)
2.38 [1.72-3.06]
Center L (14/12,027)
2.27 [1.62-2.92]
0.56 [0.22-0.89]
0.48 [0.18-0.77]
0.58 [0.29-1.05]
0.57 [0.21-0.92]
0.55 [0.14-0.99]
0.53 [0.14-0.93]
1.16 [0.56-1.78]
1.27 [0.58-1.97]
‰ [95% CI]
0 1 2 3 4 5
C
Cl‰
Overall (116/170,699)
0.68 [0.56-0.82]
Center I (20/36,442)
0.55 [0.34-0.85]
Center G (21/28,289)
0.79 [0.40-1.17]
Center E (16/22,074)
0.95 [0.59-1.45]
Center A (34/20,592)
1.49 [0.45-2.53]
Center B (5/19,738)
0.56 [0.32-0.91]
Center H (5/18,808)
0.64 [0.31-0.97]
Center C (5/12,729)
1.65 [1.14-2.31]
Center L (10/12,027)
1.62 [1.07-2.18]
0.19 [0.06-0.44]
0.21 [0.02-0.40]
0,27 [0.09-0.62]
0.25-0.02-0.48]
0.39 [0.13-0.92]
0.39 [0.05-0.73]
0.45 [0.22-0.83]
0.93 [0.33-1.54]
‰ [95% CI]
0 1 2 3 4 5

Appendix Figure 3. The number of *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* (KPC-*Kp*) isolates per number of hospital admissions among 8 healthcare centers, Italy, 2017. Centers are listed in decreasing order of number of admissions. Right column represent percent [95% CI]; red text indicates value for all centers combined; bold text indicates values standardized by age and ward of isolation. A) Prevalence of KPC-*Kp* per per 1,000 admissions (Ptot‰); B) cumulative incidence of acquired KPC-*Kp* infections among hospitalized patients (Pinf‰); and C) cumulative incidence of acquired KPC-*Kp* infections occurring >48 hours of hospital admission among hospitalized patients (CI‰). Bars show median (squares) and 95% CI for each center. Empty squares identify crude and black squares standardized data by age and ward of isolation. Vertical solid lines represent the value of prevalence/cumulative incidence obtained for all centers combined; vertical dotted lines represent the lowest and highest values obtained for the estimates of 95% confidence intervals.